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# Microstimulation of the Lumbosacral Spinal Cord: Mapping

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## Quarterly Progress Report #2

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## ABSTRACT

The objectives of this research are to define the anatomical locations of neuronal populations involved in control of genitourinary and motor functions, and to determine the physiological effects of stimulation of these neuronal populations. During this quarter we have made progress on both of these objectives. Experiments using the retrograde transneural tracer psuedorabies virus injected in the bladder and urethra of male rats were continued, and experiments using the transganglionic retrograde tracer cholera toxin β-subunit were conducted in male rats and cats. A transducer and associated electronics to measure the torque at the cat knee joint were designed, fabricated, and tested. Experiments to map the physiological responses in the bladder and urethra of microstimulation of the sacral spinal cord in male cats were continued. The knee torques generated by microstimulation of the lumbar spinal cord were also investigated.

## INTRODUCTION

Electrical stimulation of the nervous system is a means to restore function to individuals with neurological disorders. The objective of this project is to investigate the feasibility of neural prosthetics based on microstimulation of the spinal cord with penetrating electrodes. Specifically, we will use chemical and viral retrograde tracers, stimulation mapping, and field potential recordings to determine the locations in the spinal cord of the neuronal populations that control genitourinary and motor functions in the male cat. We will use selective microstimulation with penetrating activated iridium microelectrodes to determine the physiological effects of stimulation of different neural populations. The results of this project will answer fundamental questions about microstimulation of the spinal cord, and lead to development of a new generation of neural prosthetics for individuals with neurological impairments. During the second quarter of this contract we made progress on anatomical mapping of the innervation of the genitourinary organs, and on measuring the effects of microstimulation of the sacral spinal cord. We also developed and tested a knee torque transducer to be used in future microstimulation experiments to determine the responses in the motor system to microstimulation of the lumbar spinal cord. Below each of our accomplishments is summarized.

## PROGRESS IN THIS QUARTER

I. Anatomical Tracing of Genitourinary Innervation

The objective of these experiments is to determine the anatomical location and extent of the first- and higher-order neurons innervating the genitourinary system, including the bladder, urethra, and pelvic musculature.

We conducted additional tracing studies using psuedorabies virus (PRV) to complete our tracing of the CNS innervation of the bladder and urethra in male rats. PRV is a transganglionic, transneuronal retrograde tracer that infects first- and higher-order neurons after injection into the end organ, and can be detected by immunocytochemistry. Shorter term survival (3-4 day) studies using psuedorabies virus injected into the bladder or urethra were conducted to compare spinal labeling in short-term and long-term survival animals. In long-term survival animals (7-8 days) we noted a proliferation of glial cells around infected spinal neurons, and found that the infected neurons appeared to be undergoing cytolysis. Our working hypothesis is that at longer survival times, glia break down and remove infected neurons in the spinal cord, and that this will result in less labeling. The short-term survival experiments will allow us to test this hypothesis, and will provide a clear time course of the infection of spinal and higher-order neurons. The tissue from the short term injections is presently in histological processing.

We also conducted experiments with injections of cholera-toxin β-subunit (CTB), a transganglionic retrograde tracer. CTB is not carried transneuronally, as PRV is, and the results of these experiments will enable us to distinguish first- and higher-order spinal neurons in studies employing PRV. Injections of CTB were made in the bladder, pre-prostatic urethra, and penile urethra of male rats. After 3-4 days survival the rats were perfused and the spinal cords recovered

for sectioning. The tissue from these injections is presently in histological processing. In cats, injections of CTB were made into peri-urethral musculature at locations corresponding to the peak of the urethral pressure profile (~ 6cm from the tip of the penis). After 24 hours the animals were perfused and the urethral tissue preserved. These experiments will enable us to determine the end organ labeling under different injection protocols so that the pattern of neuronal labeling can be assigned to particular urethral musculature.

We have submitted an abstract to the Annual Meeting of the Society for Neuroscience reporting on our tracing experiments.

II. Strain Gage Instrumented Torque Beam and Bridge Amplifier

We designed, fabricated, and tested a strain gauge instrumented beam to measure the torque generated about the knee joint by microstimulation of the spinal cord. The beam uses 4 strain gauges in a full bridge configuration to measure the strain generated by a force applied to the beam, and was designed to be insensitive to off-axis and torsional loads. By multiplying the measured force by the moment arm (from the point of force application to the rotation center of the knee joint) the torque about the knee joint is determined. We also designed and fabricated an amplifier that provides bridge excitation voltage, signal amplification, and filtering. The transducer was calibrated and bench-tested.

***Methods*** The beam and shank holder (fig. 1A) were machined from 2014-T6 aluminum, and anodized to prevent corrosion. Four strain gages (WK-13-250BG-350, Measurements Group Inc., Raleigh, NC) were bonded to the beam to measure tensile and compressive strain. A circuit to provide bridge excitation, amplification, and filtering (fig. 2) was built around an Analog Devices 1B31 bridge amplifier chip. For this application the excitation voltage was set at 10V, the gain was left adjustable via a 10-turn precision potentiometer, and the cut-off frequency of the low pass filter was selectable with a 4-position switch from 20Hz, 50Hz, 100Hz, and 200Hz.

***Results*** The transducer was calibrated and tested by applying known masses to load different axes. Figure 1B shows the output voltage as a function of the "extension" load applied to the center of the beam and the load applied to the shank holder (i.e., loads that also produced torsion in the beam). These results demonstrate that the response of the sensor is linear (0.74 mV/g) and that it is insensitive to torsional loads. Similar results were found for loads applied in the opposite direction ("flexion"). Further testing indicated that the sensor is insensitive (<0.01 mV/g) to off-axis loads whether applied perpendicular to the gages (abduction, adduction) or parallel to the long axis of the beam. The drift of the sensor is negligible in both the unloaded (< 1g/hr) and loaded (100g, <1g/hr) conditions. These results demonstrate that the sensor performs as designed and is suitable for measurement of the torque generated at the cat ankle joint.

III. Microstimulation of the Sacral Spinal Cord in Male Cats

During this quarter we conducted 3 microstimulation experiments designed to measure the responses in the bladder and urethra to microstimulation of the sacral spinal cord.

***Methods*** Male cats were pre-anesthetized with Ketamine HCl (30-35 mg/kg, IM), a venous catheter was inserted in the cephalic vein, and anesthesia maintained with α-chlorolose (60 mg/kg IV, supplemented at 10-15 mg/kg). An abdominal incision was used to expose the bladder which was cannulated with either two PE tubes, one for emptying and filling the bladder and one for measurement of bladder pressure, or a concentric double lumen catheter. The ureters were tied, transected, and drained externally. A laminectomy was made from L5 to S1 to expose the caudal lumbar and sacral cord. The animal was mounted in a spinal frame with pins at the hips, the head in a headholder, and an L4 vertebral clamp. Body temperature was maintained between 37° and 39° C with a heat lamp, warm 5% dextrose saline with 8.4 mg/cc sodium bicarbonate added was administered IV (~20 cc/hr), and heart and respiratory rate were monitored throughout the experiment.

The pressures generated in the bladder were measured using a solid state pressure transducer connected to the superpubic PE catheter (Deltran DPT-100, Utah Medical Products, Midvale, UT). In 1 case, urethral pressures were measured using a PE tubing catheter (o.d.=0.96 mm) connected to a solid state pressure transducer. In the other 2 cases, urethral pressures were

with the mounting bar at the rostral end of the beam aligned with the knee joint rotation center and the knee and hip angles set to ~90°. Responses to 1-5s trains of various amplitude current pulses at 2-20Hz were recorded at different depths along each penetration (increment=100-400μm).

***Results*** Extension torques at the knee were generated by stimulation in both L5 and L6. Knee torques were generated by stimulation within the dorsal part of the spinal cord at more medial locations, and by stimulation within the ventral part of the cord at more lateral locations. The responses in these two regions could be further distinguished by their responses to different frequency trains. In the ventral locations the torque response followed stimulus frequencies between 2Hz and 20Hz, suggesting direct activation of motoneurons. In dorsal locations, the response was strongly dependent on stimulus frequency, suggesting transynpatic activation. An exemplar trace of the torque as a function of time is shown in fig. 1C and a recruitment curve of torque as a function of stimulus amplitude at three different depths is shown in fig.1D. These results demonstrate that the sensor is capable of in vivo measurements of the torque generated at the cat ankle joint.

Several factors confounded the results of this preliminary experiment. First, as described above, the electrodes used in this experiment bent during penetration of the cord. Thus, there is some ambiguity as to the location of the electrode during each penetration. We are presently evaluating different electrode designs and will try MicroProbe electrodes with different shank shapes. Second, the femur was not rigidly fixed during this experiment. This created 2 problems: torques generated at the hip would be registered by the sensor as knee torques, and large torques generated at the knee during root stimulation may cause some movement of the animal within the spinal frame. We have designed and implemented a fixture to secure the femur to the rigid frame in future experiments. Thirdly, torque records at different joint angles indicated that there was some degree of co-contraction occurring. Since we measured only the net isometric torque, the level of co-contraction cannot be determined directly from the records. This will be addressed in future experiments by recording electromyograms from the muscles acting at the knee.

## PUBLICATIONS

During the second quarter we submitted 3 abstracts reporting on work conducted under the contract.

1. B. Erokwu, W.M. Grill, and M.A. Haxhiu "Supramedullary innervation of the urethra", submitted to the 26th Annual Meeting of the Society for Neuroscience.
2. W.M. Grill and N. Bhadra "Genitourinary responses to microstimulation of the sacral spinal cord", submitted to the 26th Annual Meeting of the Society for Neuroscience.
3. W.M. Grill "Effects of tissue electrical properties on neural excitation", submitted to the 18th Annual International Conference of the IEEE Engineering in Medicince and Biology Society.

## OBJECTIVES FOR THE THIRD QUARTER

I. Anatomical Tracing of the Genitourinary System

We will complete analysis of the tissue from rats injected with psuedorabies virus (PRV) or cholera toxin b-subunit (CTB), and prepare a manuscript for submission for publication. We will continue tracing experiments in male cats using PRV and CTB to identify the location and extent of the first- and higher-order innervation of the urethra and urethral musculature.

II. Microstimulation Experiments

During the third quarter we will concentrate our efforts on analysis of data from microstimulation experiments conducted during the first and second quarters. This will include histological reconstruction of electrode tracks in tissue sections.

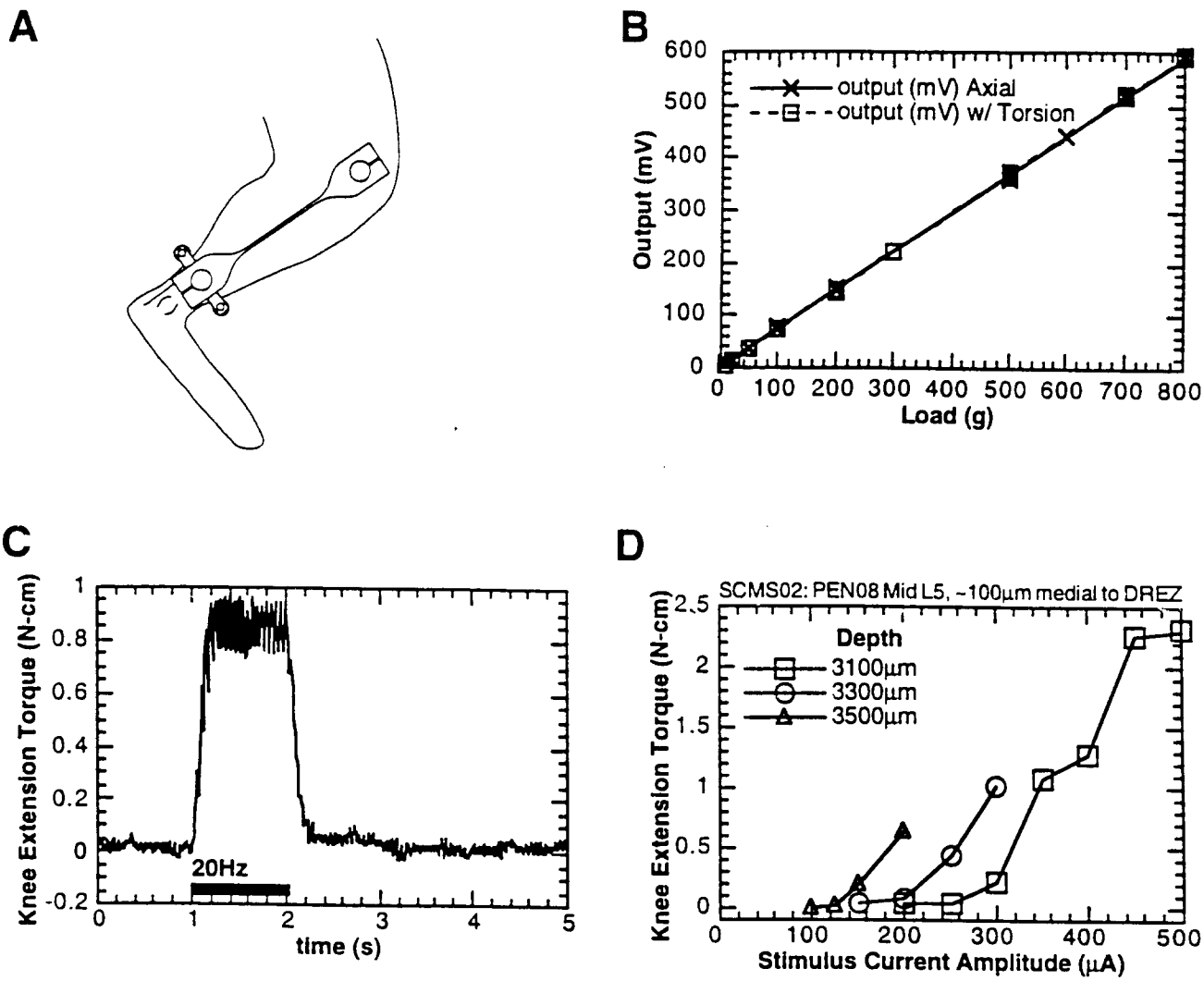


Figure 1: Strain gage instrumented beam used to measure torques at the cat knee joint generated by microstimulation. A. The beam measures the force generated at the shank using 4 strain gages in a full bridge configuration. B. Calibration curve for the beam loaded with and without torsion. C. Example of a torque response generated by microstimulation of the L5 spinal cord. D. Recruitment curves of knee torque as a function of the stimulus current amplitude (100µs, 20 Hz, 1s). The torque was quantified by the average torque during the stimulus interval because of ripple in the torque trace at 20 Hz. Note that because the electrode was bending the depths are only approximate.